BIKEN'S JOURNAL 2, 113-127 (1959)

Studies on the immunological variation in *Trypanosoma gambiense* (serotypes and the mode of relapse).

HUMIO OSAKI*

Department of Parasitology, Research Institute for Microbial Diseases, Osaka University, Osaka

(Received for publication, September 30, 1959)

SUMMARY

Twenty four antigenically different serotypes of *Trypanosoma gambiense*, the original and 23 relapsed variants, were obtained in the course of passage in mice.

An incubation of 2~3 days was very commonly observed in initial infections and the term could be protracted up to 7 days. On the other hand, the incubation time in relapses was usualiy between 5 and 9 days after recovery showing rather a scattered distribution up to 20 days. Neither first relapses before 3 days nor second and further relapses in the same mouse after 15 days have been experienced.

Serotypes induced from the first relapses of each type were mostly 3 or 4 in number and none of the same type have been seen in serotypes induced from the second and further relapses in the same mouse. Serotypes O, R_1, R_2 and R_3 were exceedingly frequent during the relapses (65.2 per cent).

Very few relapses (2.96 per cent) contained combined serotypes* but serotypes of subsequent relapses from these relapsed strains were not combined any more with an exception.

Serotypes of relapsed variants from combined infections** were independent of those of inoculated types.

The immune period to homologous serotype in mice lasted for as long as 521 days after recovery. Neither congenital transmission nor congenital immunity were seen.

Induction of variations was accomplished by two methods *i.e.* heat-treatment of infected mice and intraperitoneal or intravenous injection of immune plasma.

The variation system O→R→O was found both *in vivo* and *in vitro* but the latter half of the system *in vivo* sometimes took place by 2 or 3 step convergence.

The site and nature of these antigenic changes will be discussed.

---

* Present address: *Department of Pharmacology, Osaka University Dental School.*

Part of the studies described in this paper were supported by grants from the Ministry of Education.

The major part of this work has already been presented by the author at: the Annual Meeting of the Society of Japanese Parasitologists in 1951~1955, the Kinki Regional Meeting of the Society in 1951 (twice), 1952 and 1953 (twice), the West Japan Regional Meeting of the Society in 1955 and the Annual Meeting of the Genetics Society of Japan in 1951~1955.

The symbols "*T.g.*", "O" and "$R_{1, 2, \ldots, 23}$" designate *Trypanosoma gambiense*, original type and relapse types and R_1 and R_2 are not identical with those described in the earlier paper (Inoki *et al.*, 1952a).

* Combined serotype: a serotype consisting of two or more different serotypes.

** Combined infection: infection caused by two or more antigenically different *T.g.*.

INTRODUCTION

In earlier papers Inoki (1952) and Inoki *et al.* (1952a, 1952b, 1956, 1957) have demonstrated a new variation system of *T. g.* in mice utilizing new experimental methods by the agglomeration test between immune plasma and the organisms in the infected mouse blood. The *in vitro* method was used to see if the parasite had changed its serotype in a short time which did not permit fission.

Attention has been focused on the origin and nature of variation and the mode of relapse.

The present paper is on the variation and fates of this immunological or chemotherapeutical host-parasite relationship. Further results will be reported later.

MATERIALS AND METHOD

1. *T.g.*: Type O organisms were maintained in the laboratory in mice by serial passage for more than 1000 generations since they were received from the Institute of Infectious Diseases, Tokyo University. Antigenically different variants $R_{1, 2, \ldots, 23}$ were made during further passages without suffering any antigenic change.

2. *Experimental animals:* 4369 mice, male and female inbred strains and commercial albino mice of 12 to 17 g body weight, and hamsters were used. Rabbits were also used to get immune plasma.

3. *Dilution of blood and plasma:* Sodium citrate saline, physiological salt solution, Tyrode, glucose salt solution and Ringer were used initially. Later only physiological salt solution was used.

4. *Therapeutic agents*: Human blood plasma was mainly used. At first inactivated serum taken from samples for the serum reaction was used. Later plasma was collected from samples for the erythrocytes sedimentation reaction. Stibnal (0.3 per cent glucose), Acriflavin (0.5 per cent solution) and freeze-dried plasma were also used.

5. *Passage:* A suitable amount of blood, taken from the tail of the infected mouse was injected intraperitoneally or subcutaneously into a healthy mouse. The amount used varied according to the condition or grade of infection. The next passage was usually available after 40~60 hours. In general, a grade of infection such that one drop of venous blood from the tail contained 1~2 parasites in a microscopic field under 400 × magnification was convenient. The infected animal was then treated according to the infection and the experimental purpose.

6. *Therapy:* 0.005~0.3 ml of human glood plasma, 0.01~0.5 ml of Stibnal, a slightly smaller dose of Acriflavin than the dose of human blood plasma and 0.02~0.2 ml of freeze-dried plasma were given. More than 0.4 ml of human blood plasma caused a considerable decrease in frequency of relapses.

Parasites in peripheral blood disappeared once, usually 6~20 hours after the administration of human blood plasma, freeze-dried plasma and Acriflavin. The time of disappearance varied from 40 minutes to 10 hours with Stibnal.

The author standardized the experimental definition of "recovery" on the basis of this disappearance of the parasites from the circulating peripheral blood. The reappearance of the parasites in the peripheral blood of the same animal is regarded as "relapse".

Animals were observed every one or two days or sometimes several times a day in order to detect the slightest appearance of parasites.

7. *Collection of immune plasma*: Blood was collected by the capillary technique or by decapitation at the period having the highest agglomeration titre. This was usually 4~7 days sometimes 10~14 days (in cases of slight infection) or exceptionally 21~25 days (in case of latent infection or spontaneous recovery) after the application of the therapeutic agents.

The same or a double volume of 1～2 per cent sodium citrate in physiological saline was added and immune plasma was separated by centrifugation.

Separated plasma was stored in normal or ampulated capillaries or freeze dried and stored in a refrigerator.

Rabbits were also immunized by the vaccine method and the plasma obtained was stored in the same way.

Stored plasma was suitable for identification of serotypes and the characteristic specifity of each serotype did not change during at least six years storage although the titre gradually became weaker.

8. *Screen test:* Cured mice were completely protected against reinfection by the identical or homologous strain but not against antigenically different strains.

Since this was invariable, the method was applicable to the identification of serotypes, and the differentiation of certain strains in cases of multiple infection. A strain derived from these crosses should be antigenically different from an other (others) and it was useful to link this method with the agglomeration test for confirmation of each step of the experiment.

RESULTS

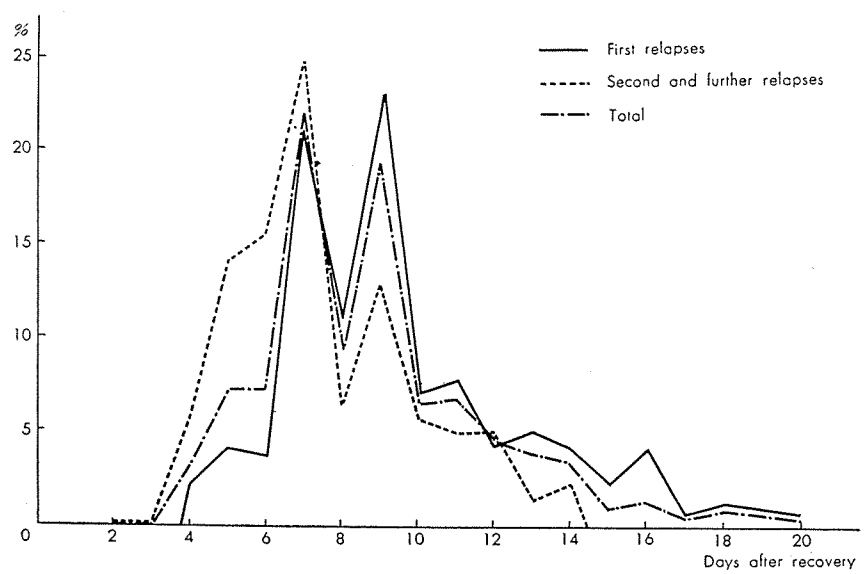
1. *Incubation:*

A. *Infection:*

Parasites usually appear in circulating peripheral blood 1, 2 or 3 days after inoculation and the term can be prolonged to 7 days by dilutions containing only a few parasites. On infection of only one parasite, the manifestation appeared between the 5th and 7th days. If the incubation was more than a week the serotype differed from that of the inoculated parasites, and the author takes it as a relapse from a latent infection or spontaneous recovery.

In hamsters, parasites appear usually in 3～5 days for 2 or 3 days or 1～2

Fig. 1 INCUBATION TERM OF RELAPSES



weeks. Parasites disappear from the circulating peripheral blood once for several days but later reappear as antigenic variants. Under some circumstances the initial manifestation lasts from several days to over a month without much variation in the number of parasites. Then there is a sudden increase in the number of parasites causing death.

The possible reappearance of the same serotype during these repetitional relapses in hamsters has not been studied.

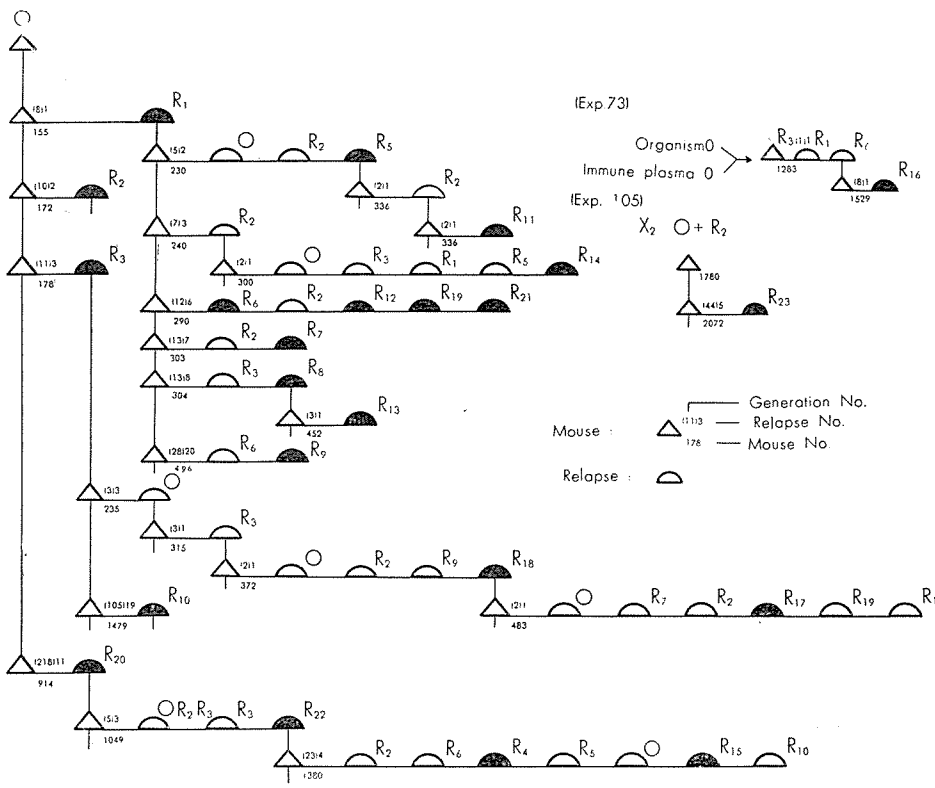
A. *Relapse:*

As shown in Figure 1, incubation varies from 2 to 20 days and 79.8 per cent of 440 relapses were seen between 5 and 11 days after recovery. There is a high frequency of the first relapses on the 7th, 8th and 9th days (55.2 per cent) and no case of a relapse before the 3rd day. Second and further relapses in the same mouse however occured mostly between the 5th and 9th days (73.0 per cent) and there was no relapse after 15 days.

The grade of infection and the therapeutic dose (below a certain level) do not appear to be correlated with the incidence of relapse or the incubation term.

Spontaneous recovery was rare. On recovery there were no or very few parasites in the peripheral blood. This period was usually followed by the

Fig. 2 FIRST INCIDENCE OF EACH TYPE SHOWN BY GENEALOGICAL TREES



appearance of different serotypes after 7 to 10 days incubation.

The effect of variations in the environment such as temperature, humidity and food on the frequency of relapse and the length of the incubation time, have not been studied.

2. *Variety of relapse:*

A. *Total number of relapses followed:*

During the study of passages and experiments on relapses from O and R, many antigenically independent serotypes were separated. Thus the original serotype and 23 relapse forms were found in the aggregate. On rare occasion (2.96 per cent) some combined serotypes containing two or more different types were found. Figure 2 shows genealogical trees of the first incidence of each type.

B. *Serotypes induced from the first relapse of each type:*

There was some regularity in the number and dominance of types appearing at the first relapse. There were not more than 6 variants of each type and usually 3 or 4 and O, R_1, R_2 and R_3 were particularly dominant. Table 1 indicates the serotypes of the first relapse in 440 cases.

The nature of the occasional incident of some dominance of certain types appearing at relapses for months is not yet clear.

Tab. 1 SEROTYPES OF FIRST RELAPSES FROM EACH OF LEADING TYPES

Relapse type / Primary type	O	R_1	R_2	R_3	R_4	R_5	R_6	R_{10}	R_{16}	R_{20}	Combination	Number of relapse types
O		+	+	+						+		4
R_1	+		+	+			+					4
R_2	+	+		+								3
R_3	+							+		+		3
R_4	+	+	+									3
R_6	+								+			2
R_{10}	+	+	+									3
R_{20}	+			+							+	3
R_{22}	+		+									2
R_{23}	+	+		+	+	+		+				6

C. *Serotypes induced at the second and subsequent relapses:*

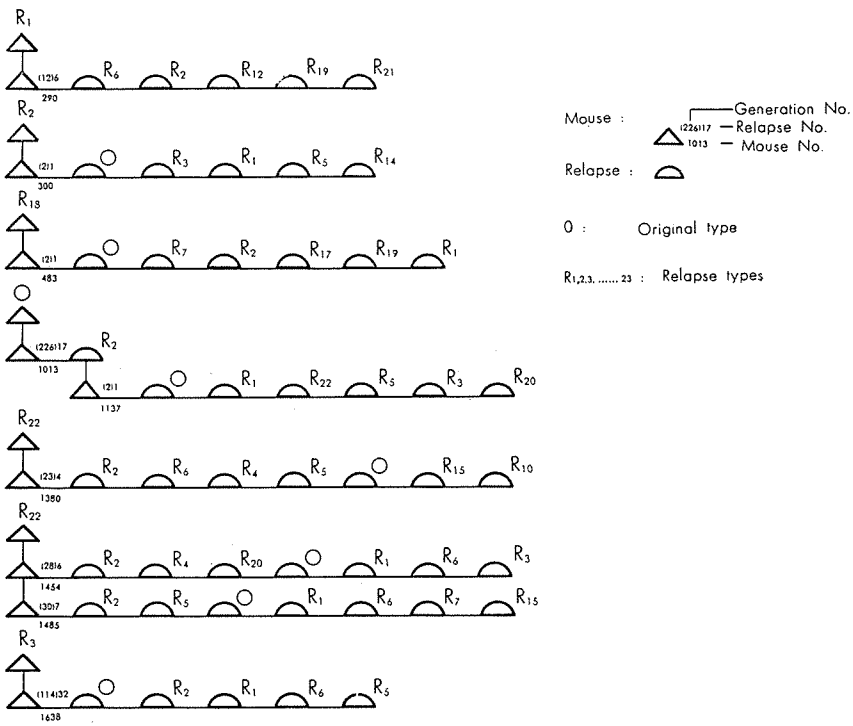
There was no case of reappearance of the same type in the same mouse up to the seventh relapse.

The frequency of appearance of the main relapse types and the pattern of repetitional relapses are demonstrated in Table 2 and Figure 3.

Tab. 2 LEADING SEROTYPES OF RELAPSES APPEARING DURING THE COURSE OF PASSAGE

Type of relapse / Sort of relapse	O	R_1	R_2	R_3	R_4	R_5	R_6	R_{10}	R_{16}	R_{20}	R_{23}	Total relapses
First relapse	79	35	54	45	5	5	12	6	7	13	5	299
(%)	(26.7)	(11.4)	(18.1)	(15.0)	(1.7)	(1.7)	(4.0)	(2.0)	(2.3)	(4.3)	(1.7)	
Second and further relapses in the same mouse	10	17	33	14	3	9	12	2	0	2	1	144
(%)	(7.1)	(12.1)	(23.4)	(9.9)	(2.1)	(6.4)	(8.5)	(1.4)		(1.4)	(0.7)	
Total	89	52	87	59	8	14	24	8	7	15	6	440
(%)	(20.2)	(11.8)	(19.8)	(13.4)	(1.8)	(3.2)	(5.5)	(1.8)	(1.6)	(3.4)	(1.4)	

Fig. 3 REPETITIONAL RELAPSES IN THE SAME MOUSE (CASES OF MORE THAN 5 RELAPSES)



3. *Two or three step convergence and some comparisons between in vitro and in vivo convergence:*

The converging variation R→O which was always found *in vitro* was not al-

ways responsible for the relapse. There was a variation in the relative difficulty of convergence. Most relapses however, which did not converge in one step had converged by the second relapse or at relapses of the second or third generations namely in two or three steps.

It was rare for relapsed parasites to show agglomeration against two or more immune plasma. However some relapses showed no significant agglomeration against any type. The serotypes of these apparently unstable variants could be identified by further passage of one or more generations.

The meaning of the differences between *in vivo* and *in vitro* procedures is not clear. The mechanism of *in vivo* change is apparently complex.

4. *Single parasite inoculation and relapse:*

Single parasites were inoculated during the passages (for example, ar the 23rd and 250th generations and three more times with O, at the 25th and 4lst generations with R_1 and at the 19th and 66th generations with R_3). There was no change in the serotype or picture of relapses.

5. *Comparison of the O→R(→O) and R→O(→R) variation systems:*

Both *in vivo* and *in vitro*, throughout the whole experiment, R→O convergence was more common than O→R divergence and O seems more stable antigenically than $R_{1,2,\text{----},23}$.

6. *Relapses of combined serotypes:*

The serotype at relapse after recovery from *T.g.* containing two or more serotypes, O and R or R and another R, was single except for one case (5.6 per cent).

7. *Resistance of parasites to human blood plasma and other therapeutic agents:*

Repeated application of therapeutic agents to infected mice tended to develop a resistant manner but it was not evident to human blood plasma. After more than 5 relapses (cf. Figures 2 and 3) in mice treated with human blood plasma, the plasma gradually became ineffective and there was a prolonged appearance or even an increase in number of parasites in the peripheral blood for two or more days after the administration of 0.3 ml of human blood plasma. Therefore the repeated treatment was not always effective.

8. *Relapse of doubled infection:*

It was of great interest, as Table 3 indicates, that serotypes of variants induced from mice which had recovered originally inoculated parasites of two or more serotypes simultaneously were always independent of those of variants from individual type inoculated.

9. *Induced variation in heat treated mice:*

The serotypes from the plasma of infected mice after exposing to high temperatures of 45°~50°C for 25~60 minutes in incubator tended to vary from those of inoculated parasites, and the hosts died, as a rule, after about 25 minutes. On the contrary, dead infected mice treated in this way showed no sign of change (see Figure 4 and Table 4).

Tab. 3 RELAPSES FROM COMBINED INFECTIONS SHOWING NO APPEARANCE OF TYPES WHICH COMMONLY APPEAR IN RELAPSES FROM EACH COMPOSITIVE TYPE OF THE INFECTIONS

Primary type \ Relapse type	O	R_1	R_2	R_3	R_6	R_9	R_{10}	R_{16}	R_{20}	R_{23}	Combination
O		+	+	+					+		
R_1	+		+	+	+						
R_2	+	+		+							
R_3	+						+		+		
$X_2=O+R_2$								+		+	$O \cdot R_3$
$X_3=R_1+R_2$						+	+		+		$R_5 \cdot R_6$
$X_4=R_2+R_3$								+			$O \cdot R_1$
$X_6=O+R_2$								+		+	$R_{16} \cdot R_{23}$

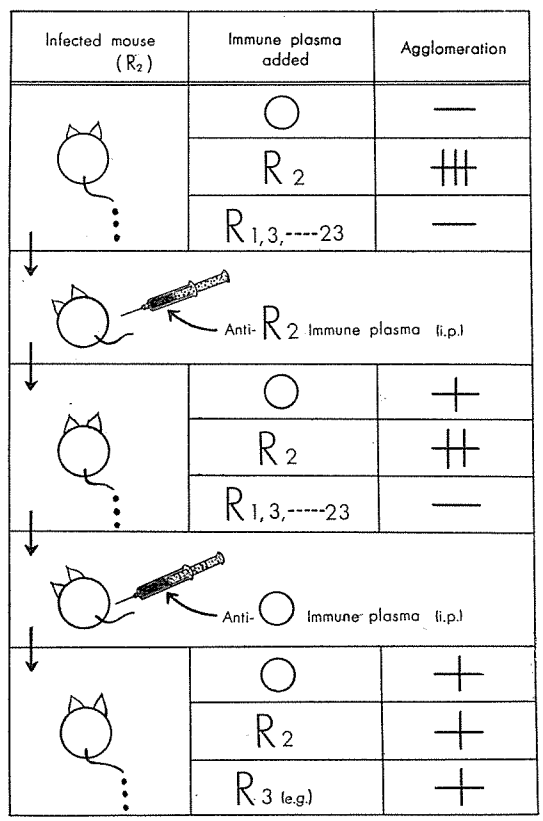
10. *The duration of immunity in mice:*

By the agglomeration test, immunity depends on the grade of infection, time or method of treatment, amount of therapeutic agent administered, as shown in Figure 5.

Although antibodies could be produced from the beginning of the infection, the existence of antibodies could have not been shown by Ehrlich's test or the screening method at any stage of infection. Very slight agglomeration tended to occur between parasites and the homologous immune plasma after the appearance of considerable numbers of organisms in the peripheral blood. However it was not caused by immune plasma potent enough to induce an antigenic variation.

After treatment, on the disappearance of the parasites from the peripheral blood, the antibody curve rose sharply to a maximum at 4~7~10 days. Then

Fig. 4 INDUCTION OF VARIATION IN MOUSE BY INJECTION OF HOMOLOGOUS IMMUNE PLASMA



Tab. 4 INDUCTION OF VARIATION IN MICE BY EXPOSURE TO A TEMPERATURE OF 50°C

Mouse	Type	Live or fatal	*T.g.*	Heat		Agglom.	
				°C	mins.	*T.g.* O	*T.g.* R_{20}
3525	R_{20}	L.	##	50	60	##	##
3531		L.	##		49	##	##
3532		L.	##		50	##	##
3534		L.	##		52	##	##
3535		L.	##		58	##	##
3536		L.	##		46	##	##
3537		F.	##		30	—	—
3544	Control	L.	Control		55	—	—

in 1∼2 months it fell gradually to a steady low level (Figure 5a). The decrease shown in results in the previous report (Inoki *et al.*, 1952a) was more rapid and this difference might be due to the more careful observations in the present work.

There was still immunity from infection by parasite of the homologous serotype 521 days after recovery (see date in Table 5). However, parasites appeared 105, 163 and 343 days after recovery in three cases, as given in Table 6, showing different but closely related serotypes. Thus immunity may be much longer.

Tab. 5 SCREENING TEST SHOWING THE DURABILITY OF THE IMMUNITY IN MICE (EXCLUDING CASES OF SINGLE SCREENING)

Serotype	Mouse No.	1	2	3	4	5	Infection
		Days after recovery	Days after recovery	Days after recovery	Days after recovery	Days after recovery	
O	3446	109	135				—
	3633	184	195				—
	4102	39	361				—
	3566	259	270				—
	3929	263	298				—
	3933	266	346	367			—
	3783	296	380				—
우유	3776	301	378	411	463	521	—

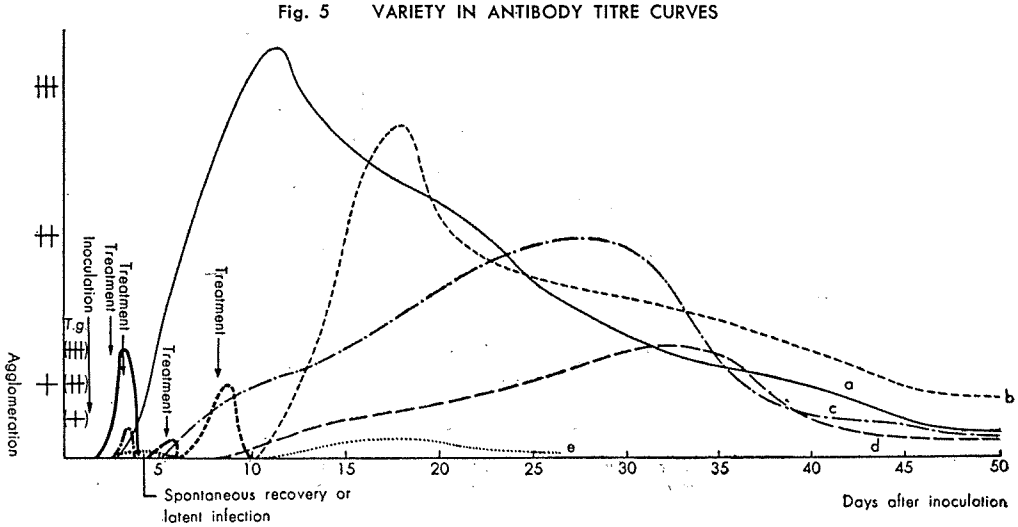
In some cases of slow infection, particularly of early treatments, antibodies were almost indetectable shortly after recovery and the rise in immunity was com-

Tab. 6 RARE CASES OF REINFECTION OF THE HOMOLOGOUS SEROTYPE ACCOMPANIED WITH VARIATION OF *T.G.* IN MICE WHICH RECOVERED

Serotype	Mouse No.	1		2		3		Days of incubation	Serotype
		Days after recovery	Inf.	Days after recovery	Inf.	Days after recovery	Inf.		
O	3428	30	−	67	−	105	+	7	R_{10}
	3703	163	+					3	R_{10}
	3713	343	+					7	R_3

paratively slow. The maximum was reached only slowly by 5 weeks (Figures 5b, 5c and 5d).

Fig. 5 VARIETY IN ANTIBODY TITRE CURVES



On spontaneous recovery, the pattern of the antibody curve was similar to that for slow infection but the peak was generally lower (Figure 5e).

Antibody was usually detectable even when the infection was latent.

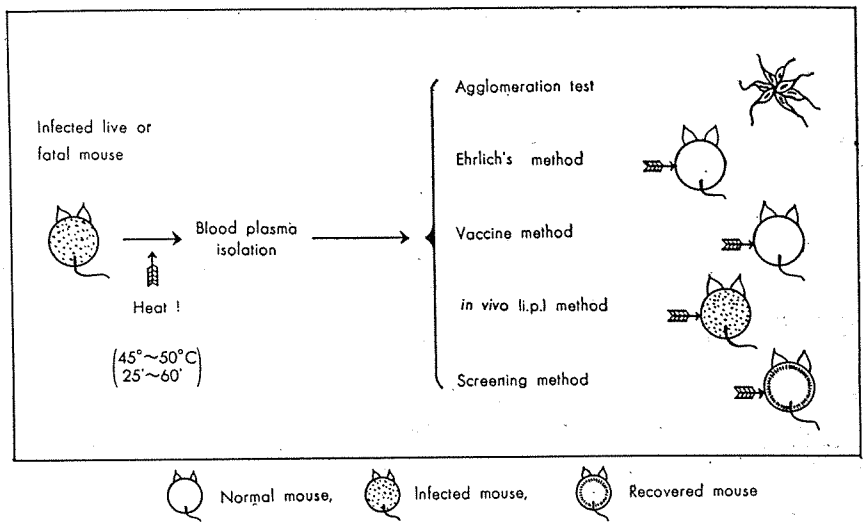
11. *Induction of in vivo variation in mice:*

Induction of *in vitro* variation of *T.g.* was described by Inoki *et al.* (1956). The variation *in vivo* was studied by intraperitoneal or intravenous injection of homologous immune plasma into *T.g.* infected mouse. Blood was taken from the tail after injection and the serotype of the parasites in the blood was found to have changed (see Figure 6 and Tables 7 and 8).

DISCUSSION

1. There is every reason to believe that some systemic transformative changes

Fig. 6 INDUCTION OF VARIATION BY HEATING METHOD OF INFECTED HOST (MOUSE)



Tab. 7 INDUCED VARIATION IN MOUSE BY INJECTION OF HOMOLOGOUS IMMUNE PLASMA INTO INFECTED MOUSE (1)

Infected mouse		R_2 (No. 2652)					
Immune plasma injected (1)		R_2 0.0025 ml (No. K5)					
Typing test (agglomeration)	mins.	Control plasma	O	R_1	R_2	R_3	R_{20}
	2	−	±	−	++	−	−
	3	−	±	−	++	−	−
	5	−	±	−	++	−	−
	13	−	±	−	++	−	−
Passage (1.)		Muse (No. 2659) 21 mins					
Immune plasma njected (2)		O 0.0025 ml (No. 1295) 27 mins					
Passaga (2.)		Mouse (No. 2660) 31 mins					
Typing test(agglomeration)	Mouse No.	Control plasma	O	R_1	R_2	R_3	R_{20}
	2659	−	±	−	++	−	−
	2660	−	±	−	+	−	+

Exp. 124

take place in protozoal diseases and these make it harder to treat or prevent the disease therapeutically.

This paper is largely on the variation in antigenic types. These are highly

Tab. 8 INDUCED VARIATION IN MOUSE BY INJECTION OF HOMOLOGOUS IMMUNE PLASMA INTO INFECED MOUSE (2)

Evp. No.	121				135			
Infected mose	R_1 (No. 2162)				R_2 (No. 3168)			
Immunse plasma injected	R_1 0.01 ml i.p. (No. 1315)				R_2 0.005 ml i.g. (No. 3044)			
Typing test (agglomeration)	O	R_1	R_2	Control plasma	Type / mins.	Control plasma	O	R_2
	—	—	##	—	Before	—	—	##
					24	±	±	##
Pnssage	Mouse No. 3174 (22 mins)				Mouse No. 3173 (26 mins)			
Typing (Exp. 121) or screening (Exp. 135) test	O	R_2	R_2	Control plasma	Mouse No. 3176 O (from)			
	—	—	##	—	--- Inhibited infection ! ---			

variable.

Ritz (1914, 1916) in *Trypanosoma brucei,* Lourie and O'Connon (1937) in *Trypanosoma rhodesiense*, Robertson (1939) in *Tetrachymena* and more recently Sonneborn (1947, 1948, 1950), Sonneborn and Le Suer (1948) and Beal (1948, 1952, 1954) in *Paramecium aurelia* have reported on the antigenic types of both pathogenic and nonpathogenic protozoa.

2. *T.g.* multiplies only by longitudinal binary division initiated by the division of the parabasal body and the "trypanosoma type" of *T.g.* can still not be cultured *in vitro.* Antigenic variation of trypanosomes may differ from that of other protozoa and bacteria.

3. Further work is required on freshly isolated parasites which are still capable of transmission through a vector or host. The present study using our standard strain, even though it has had antigenic specifity for several years through successive passages in mice, has limited significance.

4. Congenital transmission of *T.g.* in man is said to be rare but possible (Faust *et al.,* 1957). Attempts to transmit it congenitally in mice were unsuccessful.

5. Sheep, cattle, goats and pigs are regarded as potential reservoirs of parasites (Faust *et al.*, 1957) and congenital immunity must also be considerable. Tests were made to see if the offspring of animals which had recovered from *T.g.* infection were immune from infection. In two 44 day old mice born on the 419th day after recovery of the parent, no immunity was found by the screening method.

6. Repeated injection of human blood plasma to mice which had repeated relapses was sometimes found to be ineffective.

It has been observed that interrupted or otherwise irregular therapy causes the formation of drug-resistant trypanosomes. Its development and nature are not known. No attempt to explain the origin of the above ineffectiveness of hu-

man blood plasma will be made here. However if drug-resistance participates in this phenomenon it seems likely that the cell membrane of the orgenism becomes permeable to the drug. This is supported by the fact that resistant trypanosomes are said to contain less arsenic than nonresistant forms when treated with arsenicals (Eagle and Magnuson, 1944; Felsenfeld, 1944; Harding, 1945; Faust, Russell and Lincicome, 1957).

7. Spontaneous recovery with or without relapses, is possible but not general.

8. The duration of immunity was surprisingly long and constant for a protozoal infection. In three cases of infection by parasites of the homologous serotype, the mechanism of this immunity seems similar to that of the period between relapses.

9. In the course of mouse passage and experiment the author separated 24 serotypes of *T.g.*. There was some analysable order in the appearance of types at the relapse.

On treatment, all the parasites in the blood should be destroyed giving the host a long but not lifetime resistance to reinfection of antigenically homologous parasites. Even so, it is possible that some parasites are not killed but only checked. Their multiplication establishes a latent appearance as Taliaferro (1932, 1938), Packchanian (1934) and Sevag (1957) have pointed out.

Furthermore, human blood plasma especially is not trypanocidal and the mechanism of its action in the parasitized host is yet unknown.

10. The facts that repeated occurrence of relapses in the same mouse were never of the same serotype and that relapse from the combined infection by different types has prevented the appearance of nearly related serotypes are apparently contradictory. However it is conceivable that some genic or cytoplasmic antigen-determining substance controls both the above phenomena and the attenuated survivors acquire a certain serotype which is still viable. While in initial infections an incubation of 2~3 days was very common, the incubation period in relapses was mostly between 5 and 9 days after recovery.

From the above results relapses may be the result of a competition, like a hide-and-seek, based on some chemotherapeutical host-parasite relationships at a molecular level.

11. The occasional appearance of combined serotypes followed by the lead into a single serotype at next stages and the attitude of the 2 or 3 step reversal to O suggest the possible existence of sub-type and the lesser stability of R as compared to O.

12. Our new variation system of *T.g.* O→R→O held good. The latter half *in vivo* is sometimes complicated as above cited, but the reversion to O is not blocked.

13. The author refers to the result of the induction of variation in heat treated mice here not because of the possibility of attenuation of parasitic virulence by exposure to a high temperature but as an interpretation of the way in which variation occurs.

Death of the host does not participate in alteration of the serotype and heat treated *T.g. in vitro* never showed any change in serotype.

These and other results, as for example the mode of repeated relapses in the

same mouse, are interpreted to mean that the surviving parasites have been adaptively modified in their antigenic peculiarities with a result that they are not susceptible to antagonistic forces or antibodies.

Thus it is hard to know what occurs in the host during and after infection. The problem must remain unsettled until further experiments under various conditions are made to elucidate the above results.

14. The medical significance of these variations of *T.g.* are great. It is likely that the next advanee in the chemotherapy of trypanosomiasis will be (Goodwin and Rollo, 1955) in the development of completely new types of organic compounds, pərhaps of metabolic antagonists.

ACKNOWLEDGEMENT

Among the many people to whom the author is deeply indebted for constant guidance, advice and encouragement during the course of this work he would like to mention particularly Drs. Kaoru Morishita, Shozo Inoki, Toshio Nakabayashi, Suehisa Takada and Shigemitsu Fukukita.

REFERENCES

Beal, G. H. (1948). The process of transformation of antigenic type in *P. aurelia*, variety 4, *Proc. Nat. Acad. Sci., U. S.* **34,** 418–423.

Beal, G. H. (1952). Antigen variation in *P. aurelia*, variety 1, *Genetics* **37,** 62–74.

Beal, G. H. (1954). *The genetics of Paramecium aurelia*, Cambridge, Cambridge University Press, pp. 77–123.

Eagle, H. and Magnuson, H. J. (1944). The spontaneous development of arsenic resistance in *Trypanosoma equiperdum* and its mechanism, *J. Pharmacol. and exper. Therap.* **82,** 137–151.

Faust, E. C., Russell, P. F. and Lincicome, D. R. (1957). *Clinical parasitology*. 6th edition, Philadelphia, Lea & Febiger, p. 146 and p. 151.

Felsenfeld. O. (1951). *African sleeping sickness; Clinical tropical medicine*, St. Louis, The C. V. Mosby Co., p. 167.

Goodwin, L. G. and Rollo, I. M. (1955). The chemotherapy of piroplasmosis, trypanosomiasis, and leishmaniasis, in *Biochemistry and physiology of protozoa* (S. H. Hunter and André Lwoff, editors) Vol. **2,** New York, Academic Press Inc., pp. 254–268.

Harding, R. D. (1945). Late results of treatment of sleeping sickness in Sierra Leone by Antrypol, Tryparsamide, Pentamidine and Propamidine, single and in various combinations, *J. Roy. Soc. Trop. Med. & Hyg.* **39,** 99–124.

Inoki, S. (1952). A new experimental method and genetical interpretation on the antigenic variation in *Trypanosoma gambiense*, *Med. J. Osaka Univ.*, **3,** 81–86.

Inoki, S., Kitaura, T., Nakabayashi, T. and Kurogochi, H. (1952a). Studies on the immunological variation in *Trypanosoma gambiense*. I. A new variation system and a new experimental method, *Med. J. Osaka Univ.* **3,** 357–371.

Inoki, S., Kitaura, T., Kurogochi, Y., Osaki, H. and Nakabayashi, T. (1952b). Genetical studies on the antigenic variation in *Trypanosoma gambiense*, *Jap. J. Gen.* **27,** 85–92.

Inoki, S., Osaki, H. and Nakabayashi, T. (1956). Studies on the immunological variation in *Trypanosoma gambiense*. II. Verifications of the new variation system by Ehrlich's and *in vitro* methods, *Med. J. Osaka Univ.* **7,** 165–173.

Inoki, S., Nakabayashi, T., Osaki, H. and Fukukita, S. (1957). Studies on the immuno-

logical variation in *Trypanosoma gambiense*. III. Process of the antigenic variation in mice, *Med. J. Osaka Univ.*, **7**, 731–743.

Lourie, E. M. and O'Connon, R. J. (1947). A study of *Trypanosoma rhodesiense* relaping strains *in vitro*, *Ann. Trop. Med. Parasit.* **31**, 319–340.

Packchanian, A. (1934). Experimental *Trypanosoma brucei* infection and immunity in various species of peromyscus (American Deer Mice), *Am. J. Hyg.* **20**, 135.

Ritz., H. (1914). *Deutsch. Med. Wochensch*, **40**, 1355.

Ritz, H. (1916). Über Rezidive bei experimenteller Trypanosomiasis, *Arch. f. Schiffs- u. Trop. Hyg.* **20**, 377–420.

Robertson, M. (1939). A study of the reactions *in vitro* of certain ciliates belonging to the *Glaucoma-Colpidium* group to antibodies in the sera of rabbits immunized therewith, *J. Pathol.* **48**, 305–322.

Sevag, M. G. (1957). *Summary of current conceipts of resistance of the parasite; Host-parasite relationships in living cells*, Springfield, Illinois, Charles C. Thomas, p. 206.

Sonneborn, T. M. (1947). Recent advances in the genetics of *Paramecium* and *Euplotes*, *Adv. Genet.* **1**, 264–358.

Sonneborn, T. M. (1948). The determiation of hereditary antigenic differences in genetically identical paramecium cells, *Proc. Nat. Acad. Sci., U. S.* **34**, 413–418.

Sonneborn, T. M. (1950). The cytoplasm in heredity, *Heredity* **4**, 11–36.

Sonneborn, T. M. and Le Suer, A. (1948). Antigenic characters in *Paramecium aurelia* (variety 4). Determination, inheritance and induced mutations, *Amer. Nat.* **82**, 69–78.

Taliaferro, W. H. (1932). Trypanocidal and reproduction-inhibiting antibodies to *Trypanosoma Lewisi* in rats and rabbits, *Am. J. Hyg.* **16**, 32.

Taliaferro, W. H. (1938). Ablastic and trypanocidal antibodies against *Trypanosoma Duttoni*, *J. Immunol.* **35**, 303.